SYNTHESIS AND CHROMATOGRAPHIC PROPERTIES OF PARTIALLY *O*-METHYLATED 2-DEOXY-2-METHYLAMINO-D-GLUCOSES; STANDARDS FOR METHYLATION STUDIES ON POLYSACCHARIDES*

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ABSTRACT

3-, 4-, 6-Mono-, 3,4-, 3,6-, 4,6-di- and 3,4,6-trimethyl ethers of 2-deoxy-2-methylamino-D-glucose hydrochloride have been prepared. The chromatographic behaviour of these derivatives on paper and on an amino acid analyzer, and the retention times of their *O*-trimethylsilyl derivatives by g.l.c. were determined. These constants are for use in the identification of fragments arising from polysaccharides containing 2-acetamido-2-deoxy-D-glucopyranosyl residues when they are successively methylated by the Kuhn procedure and hydrolyzed.

INTRODUCTION

Structural investigations on polysaccharides, oligosaccharides, or glycoproteins containing amino sugars have frequently required the use of methylation techniques[1,2]. These methods have been summarized for the period up to 1963 by Wallenfels *et al.*[1] and up to 1966 by Neuberger and Marshall[2]. The Kuhn procedure[3], which is carried out with methyl iodide–*N*,*N*-dimethylformamide in the presence of silver oxide, has two advantages. Many polysaccharides are soluble in the reaction medium and the methylation proceeds virtually to completion. However, the acetamido groups are *N*-methylated[4] under these conditions. In this respect, the reagent differs from methyl iodide–*N*,*N*-dimethylformamide–barium oxide, which does not cause *N*-methylation. Methylation of a polysaccharide containing 2-acetamido-2-deoxy-D-glucose residues by the Kuhn procedure, with subsequent hydrolysis would, therefore, give *O*-methylated 2-deoxy-2-methylamino-D-glucose hydrochlorides. The present study describes the synthesis of 2-deoxy-2-methylamino-D-glucose hydrochloride and the three mono-, three di-, and one tri-methyl ethers that can arise from 2-acetamido-2-deoxy-D-glucopyranose residues**. The standards were compared on paper chromatograms, on a Spinco amino acid analyzer, and as *O*-trimethylsilyl (t.m.s.) derivatives by using g.l.c. Their proton magnetic resonance (p.m.r.) spectra were determined as a

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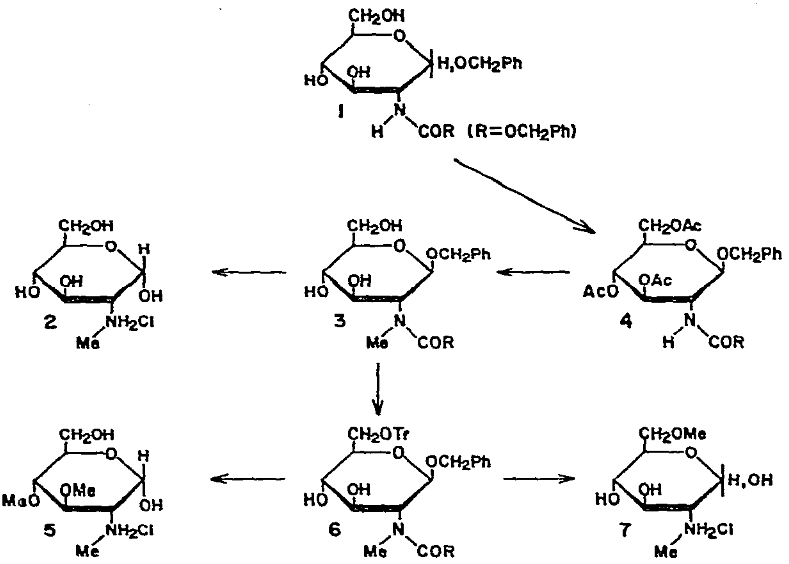
**Polysaccharides containing free amino groups can be examined in this way providing that suitable *N*-substitution, such as *N*-acetylation, is performed prior to methylation.

criterion of purity and to determine they could be differentiated. The properties thus recorded are currently being used to analyze yeast cell-wall polysaccharides[5] and glycoprotein[6] that contain residues of 2-acetamido-2-deoxy-D-glucose.

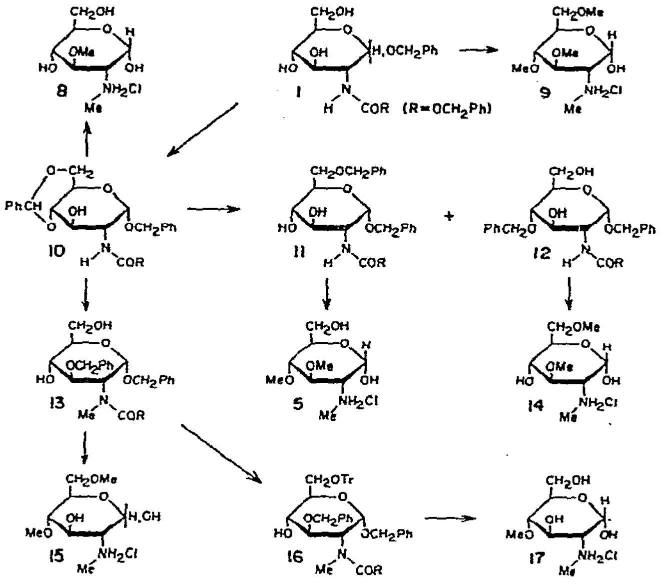
Preparation of 2-deoxy-2-methylamino-D-glucose hydrochloride derivatives. — These compounds were prepared as shown in Schemes 1 and 2. 2-Deoxy-2-methylamino-α-D-glucose hydrochloride (**2**) was prepared as follows. An anomeric mixture of benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-D-glucopyranosides (**1**) was acetylated and the triacetate (**4**) of the β-anomer isolated[7]. *N*-Methylation by the Kuhn procedure[3] followed by deacetylation gave benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-β-D-glucopyranoside (**3**). Compound **3** was hydrogenolyzed over palladium on charcoal to 2-deoxy-2-methylamino-D-glucose, which was isolated as the α-anomer of its hydrochloride (**2**).



Scheme 1. Reaction scheme for preparation of 2-deoxy-2-methylamino-D-glucose hydrochloride and its 6- and 3,4-di-methyl ethers.

The 3,4-dimethyl ether of 2-deoxy-2-methylamino-α-D-glucose hydrochloride (**5**) was prepared from **3**. The 6-trityl ether (**6**) was obtained by tritylation, purified by chromatography and then *O*-methylated and detritylated to give benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-3,4-di-*O*-methyl-β-D-glucopyranoside. This product was purified chromatographically and debenzylated by hydrogenolysis over palladium on charcoal to give the reducing amino sugar, which was converted into its hydrochloride (**5**).

2-Deoxy-6-*O*-methyl-2-methylamino-α,β-D-glucose hydrochloride (**7**) was prepared from the 6-trityl ether (**6**), which was treated with benzyl bromide and silver oxide in *N*,*N*-dimethylformamide[8] to give the 3,4-dibenzyl ether. This product was detritylated and the resulting 3,4-dibenzyl ether was purified chromatographically and *O*-methylated to give benzyl 3,4-di-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-6-*O*-methyl-β-D-glucopyranoside. Hydrogenolysis by using palladium on charcoal followed by acidification with hydrochloric acid gave 2-deoxy-6-*O*-methyl-2-methylamino-α,β-D-glucose hydrochloride (**7**).



Scheme 2. Reaction scheme for preparation of the 3-, 4-, 3,6-di-, 4,6-di-, 3,4-di-, and 3,4,6-trimethyl ethers of 2-deoxy-2-methylamino-D-glucose hydrochloride.

2-Deoxy-3,4,6-tri-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**9**) was prepared by successive Kuhn methylation and palladium-catalyzed hydrogenolysis of benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranoside (β anomer of **1**).

The remaining *O*-methylated 2-deoxy-2-methylamino-D-glucose hydrochlorides were prepared by using benzyl 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside (**10**) as starting material. Compound **10** was prepared by treatment of **1** with benzaldehyde containing zinc chloride, followed by resolution of the α,β-anomeric mixture[7].

The preparation of 2-deoxy-3,6-di-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**14**) was effected as follows. The 4,6-*O*-benzylidene ring in **10** was cleaved by using lithium aluminum hydride–aluminum chloride[9] giving a mixture of 6- (**11**) and 4-benzyl (**12**) ethers, which was resolved chromatographically. Methylation of each compound by hydrogenolysis and acidification with hydrochloric acid gave the 3,4-di- (**5**) and 3,6-di-methyl (**14**) ethers of 2-deoxy-2-methylamino-α-D-glucose hydrochloride, respectively. The two compounds were identified by comparison of their p.m.r. spectra with that of the 3,4-dimethyl ether prepared unambiguously via **6** (Scheme 1).

The 4,6-dimethyl ether (**15**) was prepared as follows. Compound **10** was *O*-acetylated by using acetic anhydride–pyridine and the resulting 3-acetate[7] was *N*-methylated by the Kuhn procedure. Following deacetylation and chromatographic purification, the *N*-methyl derivative of **10** was obtained. It was benzylated by using benzyl bromide and *N*,*N*-dimethylformamide in the presence of silver oxide. (This reagent was used since benzyl chloride–potassium hydroxide at 120° replaced the *N*-benzyloxycarbonyl group by a benzyl group). The product was hydrolyzed with aqueous acetic acid to give benzyl 3-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-α-D-glucopyranoside (**13**), which was purified chromatographically.

TABLE I

CHROMATOGRAPHIC CHARACTERISTICS OF 2-DEOXY-2-METHYLAMINO-α,β-D-GLUCOSES AND THEIR T.M.S. DERIVATIVES

O-Methyl derivative of 2-deoxy-2-methyl-amino-D-glucose	*Rates of migration relative to rhamnose on a paper chromatogram*	*Retention times on amino acid analyzer, relative to phenylalanine*[a], *min*	*Retention times of t.m.s. derivatives on g.l.c., min*	
			Major peak	*Minor peak*
Unsubstituted	0.40	−6[b]	12.4 (76%)	20.4 (24%)
3-	0.60	−31	9.4 (81%)	12.8 (19%)
4-	0.68	−18	7.3 (73%)	12.3 (27%)
6-	0.68	−11[b]	13.8 (78%)	16.6 (22%)
3,4-Di-	0.85	+40	6.5 (84%)	9.4 (16%)
3,6-Di-	0.89	−40	10.4	—
4,6-Di-	0.93	+8	8.1 (80%)	10.6 (20%)
3,4,6-Tri-	1.23	+55	7.3	—

[a]Retention time of internal standard (10 μg), 192 min. [b]These materials are virtually inseparable, since they have a peak width of 13 min compared with 6 min for phenylalanine. They are, however, distinguishable by g.l.c. of their t.m.s. derivatives.

TABLE II

CHEMICAL SHIFTS OF CERTAIN SIGNALS IN THE P.M.R. SPECTRA OF *O*-METHYLATED 2-DEOXY-2-METHYL-AMINO-α,β-D-GLUCOSE HYDROCHLORIDES IN DEUTERIUM OXIDE

Methyl ether of 2-deoxy-2-methyl-amino-D-glucose hydrochloride	*Chemical shift of* OCH_3 *signals, τ*	*Chemical shift of* NCH_3 *signals, τ*		*Chemical shift of H-1 signals, τ*	
		α-Anomer	*β-Anomer (minor)*	*α-Anomer* $J_{1,2}$ *4 Hz*	*β-Anomer (minor)* $J_{1,2}$ *8 Hz*
Unsubstituted	—	6.72	6.69	3.97	4.48
3-	5.89	6.74	6.70	3.98	4.48
4-	5.95	6.73	6.70	3.99	4.52
6-	6.16	6.75	6.71	4.01	4.52
3,4-Di-	5.88, 5.94	6.75	6.71	4.00	4.51
3,6-Di-	5.90, 6.15	6.75	6.71	4.00	4.49
4,6-Di-	5.98, 6.15	6.75	6.72	4.02	4.52
3,4,6-Tri-	5.87, 5.96, 6.13	6.75	6.71	4.01	4.51

Methylation followed by hydrogenolysis and acidification with hydrochloric acid gave 2-deoxy-4,6-di-*O*-methyl-2-methylamino-α,β-D-glucose hydrochloride (**15**).

2-Deoxy-3-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**8**) was prepared straightforwardly from **10** by methylation according to the Kuhn procedure, which caused *O*- and *N*-methylation, followed by hydrolysis of the *O*-benzylidene group, debenzylation, and acidification with hydrochloric acid.

2-Deoxy-4-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**17**) was prepared as follows. The 3-benzyl ether **13** was tritylated to give **16**, which was methylated and the trityl group removed. (Column chromatography was used to purify the intermediates at the first and third steps). The product was debenzylated and the reducing sugar acidified with hydrochloric acid to give **17**.

*Chromatographic properties of 2-deoxy-*O*-methyl-2-methylamino-D-glucoses and derivatives.* — Three main methods can be used for differentiating *O*-methylated 2-deoxy-2-methylamino-D-glucose hydrochlorides following their formation as the end-products of methylation–fragmentation experiments. These are by paper chromatography, by g.l.c. of their t.m.s. derivatives, and by their behaviour on a Spinco amino acid analyzer. In addition, their p.m.r. spectra exhibit *O*-methyl signals of distinctive chemical shift, as shown in Table II (the NMe and H-1 signals show little variation of shift). However this method of identification suffers from the disadvantages of requiring isolation of pure material in at least milligram quantities.

Very small quantities, of the order of a few micrograms of the methyl ethers, can be identified by g.l.c. of the t.m.s. derivatives* (Table I), providing that prior removal of excess contaminating material by another chromatographic method, such as paper chromatography or cellulose-column chromatography, is carried out. The most likely contaminants that could interfere with g.l.c. are the di-*O*-, tri-*O*-, and tetra-*O*-methyl-hexoses, which migrate faster on paper than the *O*-methylated 2-deoxy-2-methylamino-D-glucoses. The only exception is the 3,4,6-trimethyl ether of the latter, which has an R_F value close to those of di-*O*-methyl-hexoses[5]. G.l.c. of the t.m.s. derivatives of equilibrated α,β-anomeric mixtures of the *N*-methylated sugars gives rise, in six of eight cases, to two peaks. With the other two cases only one peak is formed. The major peaks, for the α-anomers, have lower retention times than their β-counterparts (Table I). The peaks of t.m.s. derivatives were assigned to α anomers with 2-deoxy-2-methylamino-α-D-glucose hydrochloride and its 3- and 4-monomethyl and 3,4-dimethyl ethers, since they were formed exclusively on trimethylsilylation of the crystalline α-anomers of the amino sugars. The peaks having lower retention times were formed predominantly from the amorphous 6-monomethyl and 4,6-dimethyl ethers, which exist, according to p.m.r. spectroscopy of solutions in deuterium oxide, in mostly the α-forms. Attempts to separate the acetates[13] of the *N*-methylated alditols by g.l.c. were not successful, presumably because of decomposition on the column.

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*T.m.s. derivatives of hexosamines prepared similarly are not *N*-silylated[10,11,12] to an appreciable extent.

Larger quantities of *O*-methylated 2-deoxy-*O*-methyl-2-methylamino-D-glucose hydrochlorides are required for paper chromatography in butyl alcohol–ethanol–pyridine–water with the ninhydrin spray. This technique, although capable of separating the mono-, di-, and tri-methyl ethers as groups is ineffective in separating the individual mono- and di-methyl ethers (Table I).

Methyl ethers of 2-deoxy-2-methylamino-D-glucose can be distinguished by their retention times on a Spinco amino acid analyzer[14], which records at 570 nm, the absorption maximum of the color formed by reaction of ninhydrin with the sugars in this series. Those not differentiated are 2-deoxy-2-methylamino-D-glucose and its 6-methyl ether. These can, however, be distinguished by g.l.c. of their t.m.s. derivatives. The retention times on the analyzer are quoted in Table I relative to the retention time of an internal standard of phenylalanine. From 0.2–1.0 mg of amino sugar is required for this technique. Both the paper-chromatographic and the amino acid-analyzer techniques have the disadvantage that *N*-methylated amino acids arising from glycoproteins are detected simultaneously. However, with the amino acid analyzer, methylamino sugars give much broader peaks than amino acids and, in the main, have higher retention times.

CONCLUSION

Following synthesis of the *O*-methylated derivatives of 2-deoxy-2-methylamino-D-glucose hydrochlorides and measurement of some of their chromatographic characteristics it appears that they can be identified in methylation–fragmentation products of polysaccharides. In polymers containing appreciable proportions of 2-acetamido-2-deoxy-D-glucose residues[5], more than one technique can be used, leading to a positive identification, despite a few similarities of the individual properties of the *N*-methyl sugars. In polymers containing only minute proportions of 2-acetamido-2-deoxy-D-glucose residues, such as is yeast cell-wall glycoprotein[6], the investigation is limited to the sensitive g.l.c. technique for identification purposes unless experiments are conducted on a large scale and the resulting fragments concentrated by fractionation.

EXPERIMENTAL

General. — In order to follow column chromatography on silicic acid, t.l.c. of the eluates was carried out with Merck Silica Gel 0.05–0.2 mm (70–325 mesh ASTM) and chloroform, or chloroform containing an appropriate proportion of methanol. Spots were revealed by spraying with 50% sulfuric acid and charring the products at 120°.

Hydrochlorides of the amino sugar derivatives were separated on paper by using 40:11:2:19 (*v*/*v*/*v*/*v*) butyl alcohol–ethanol–pyridine–water as solvent and ninhydrin in acetone as spray reagent. Papers were developed for 3 min at 110° in the presence of steam.

P.m.r. spectroscopy was carried out with a Varian 100-MHz n.m.r. spectrometer in order to quantitate the H-1, *N*-methyl, and *O*-methyl signals in 2-deoxy-*O*-methyl-2-methylamino-D-glucose hydrochlorides. Tetramethylsilane in a coaxial capillary was used as external standard for spectral determinations of hydrochlorides in deuterium oxide at 25°, and as an internal standard for the other compounds in chloroform-*d*. Chemical shifts are expressed in τ values based on $\tau = 10$ for the standard.

Methylamino sugar hydrochlorides were examined by using a Model 120C Spinco amino acid analyzer[14] with the UR-30 type of resin and a running time of 250 min (see Spinco procedures manual 120C-PM-1). The column (57×0.9 cm in diameter) was operated at 55° with 0.2M citrate buffers (buffer, pH 3.28 was used for first 135 min; second buffer: pH 4.25) at a flow-rate of 65 ml/h. The ninhydrin was used in the normal way with 2-methoxyethanol. Stannous chloride and ninhydrin color-responses for the 2-deoxy-*O*-methyl-2-methylamino-D-glucoses at 570 nm are approximately one twentieth that for 2-amino-2-deoxy-D-glucose, which is in turn approximately the same as that for phenylalanine.

Methylamino sugar hydrochlorides (~ 1 mg), following equilibration in pyridine (0.1 ml) for 3 h, were converted into their t.m.s. derivatives by the method of Sweeley *et al.*[15]. The reagent (0.2 ml) was used, and the solutions (3 μl) were applied directly to a $3\,\mathrm{ft} \times 1/8$ in. (internal diameter) glass column containing 10% of neopentyl glycol sebacate on Chromosorb W[13] maintained at 140° and pretreated with Silyl 8 column conditioner (0.2 ml), Pierce Chemical Company. The g.l.c. unit was a Hewlett–Packard Model 402 incorporating a hydrogen-flame detector.

2-Deoxy-2-methylamino-α-D-glucose hydrochloride (**2**). — Benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-α,β-D-glucopyranoside (**1**) was acetylated and the crystalline triacetate (**4**) of the β-anomer isolated according to the procedure of Gross and Zimmerman[7]. A sample (1.60 g) was *N*-methylated by shaking it for 18 h in methyl iodide (5 ml) and *N,N*-dimethylformamide (5 ml) containing silver oxide (5 ml). Excess chloroform was added, and the solution, was filtered and evaporated to a syrup, which was deacetylated by 0.1M sodium methoxide in methanol (20 ml). The resulting benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-β-D-glucopyranoside (**3**) was extracted with ethyl acetate after the addition of water and crystallized from ethanol; yield 0.83 g. Following recrystallization it had m.p. 138–139°, $[\alpha]_D^{25}$ $-37°$ (*c* 0.2, acetone).

Anal. Calc. for $C_{22}H_{27}NO_7$: C, 63.30; H, 6.52; N, 3.36. Found: C, 62.90; H, 6.53; N, 3.32.

The product (0.30 g) in acetic acid (15 ml) was hydrogenolyzed over 5% palladium catalyst on charcoal (2 successive additions of 0.3 g each). After 18 h the solution was filtered, evaporated to a syrup, dilute hydrochloric acid was added, and the solution evaporated. Crystallization from ethanol–acetone gave 2-deoxy-2-methylamino-α-D-glucose hydrochloride (**2**, 0.16 g) having m.p. 157°, $[\alpha]_D^{25}$ $+99° \rightarrow +84°$ (*c* 0.2, equil., water). Kuhn and Bister[16] reported m.p. 157°, $[\alpha]_D^{22}$ $-100° \rightarrow -83°$ (equil., water) for the L-enantiomorph.

Anal. Calc. for $C_7H_{16}ClNO_5$: N, 6.10. Found: N, 6.18.

2-Deoxy-3,4,6-tri-O-methyl-2-methylamino-α-D-glucose hydrochloride (**9**). — Benzyl 2-[(benzyloxycarbonyl)amino]-2-deoxy-β-D-glucopyranoside (β-anomer of **1**, 0.50 mg) was methylated by the Kuhn procedure[3] to give benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-3,4,6-tri-*O*-methyl-β-D-glucopyranoside (0.59 g); p.m.r. data: τ 6.50, 6.58, 6.60 (3 OMe groups), 7.05 (NMe). It was chromatographed on a column of silicic acid with chloroform as eluant. Debenzylation by the procedure described for preparation of **2** gave a product that was crystallized from ethyl acetate. The resulting 2-deoxy-3,4,6-tri-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**9**, 0.21 g) had m.p. 202° dec., $[\alpha]_D^{25}$ $+122° \rightarrow +113°$ (*c* 0.2, equil., water). Barker *et al.*[17] reported decomposition in the range 170–200° and $[\alpha]_D^{20}$ +105° (equil., water).

Anal. Calc. for $C_{10}H_{22}ClNO_5$: N, 5.16. Found: N, 4.94.

2-Deoxy-4,6-di-O-methyl-2-methylamino-α,β-D-glucose hydrochloride (**15**). — Benzyl 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside (**10**), consisting mainly of the α-anomer, was prepared and converted into its 3-acetate[7]. The acetate (3.4 g) was *N*-methylated in a mixture of *N*,*N*-dimethylformamide (5 ml) and iodomethane (5 ml) containing silver oxide (3.4 g). After 18 h the solution was diluted with chloroform, filtered, and evaporated. The residue was dissolved in 0.1M methanolic sodium methoxide (30 ml) and after 1 h the solution was evaporated and the residue partitioned between ethyl acetate and water. The ethyl acetate portion containing benzyl 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-α-D-glucopyranoside was evaporated and the residue *O*-benzylated overnight in a mixture of *N*,*N*-dimethylformamide (5 ml) and benzyl bromide (5 ml) containing silver oxide (5 g). The solution was diluted with excess chloroform, filtered, and evaporated. Since some difficulty was experienced in removing the last traces of solvent, the mobile liquid obtained after evaporation at 100° (with the aid of a vacuum pump) was dissolved in acetic acid (30 ml) containing water (10 ml). The *O*-benzylidene group was removed by heating for 1 h at 100°. The solution was evaporated and the residue fractionated on a column of silicic acid. Chloroform eluted unwanted material, and chloroform containing 2% of methanol eluted benzyl 3-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-α-D-glucopyranoside (**13**, 2.1 g) in advance of a trace of the β-anomer. Two crystallizations from ether gave compound **13**, m.p. 136–137°, $[\alpha]_D^{25}$ +121° (*c* 0.2, acetone).

Anal. Calc. for $C_{29}H_{33}NO_7$: C, 68.62; H, 6.55; N, 2.76. Found: C, 68.29; H, 6.68; N, 2.94.

Treatment with benzaldehyde–zinc chloride gave the 4,6-benzylidene acetal having m.p. 131° (from ethanol–hexane), $[\alpha]_D^{25}$ +65° (*c* 0.2, acetone).

Anal. Calc. for $C_{36}H_{37}NO_7$: C, 72.59; H, 6.26; N, 2.35. Found: C, 72.69; H, 6.39; N, 2.32.

Methylation of compound **13** (0.52 g) by the Kuhn procedure gave the 4,6-dimethyl ether (0.59 g), p.m.r. data: τ 6.27, 6.57 (2 OMe groups), 7.03, 7.13 (1 NMe). The product was purified by column chromatography on silicic acid with chloroform as eluant and hydrogenolyzed over palladium on charcoal to give 2-deoxy-4,6-di-*O*-methyl-2-methylamino-α,β-D-glucose hydrochloride (**15**). Chromatography on a cellulose column (eluant: acetone–water, 9.1 v/v) gave an amorphous product (0.22 g) having $[\alpha]_D^{25}$ +83° (*c* 0.2, water).

Anal. Calc. for $C_9H_{20}ClNO_5$: N, 5.44. Found: N, 5.22.

2-Deoxy-3,4-di-O-methyl-2-methylamino-α-D-glucose hydrochloride (**5**) *and 2-deoxy-3,6-di-O-methyl-2-methylamino-α-D-glucose hydrochloride* (**14**). — Benzyl 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside[7] (**10**, 3.00 g) was dissolved in 1:1 dichloromethane–ether (150 ml) and lithium aluminum hydride (1.0 g) was added. Aluminum chloride (3.0 g) in ether (60 ml) was added dropwise to the magnetically stirred solution over a period of 1 h and the mixture was boiled under reflux overnight. Excess reagent was decomposed with acetic acid and the solution shaken successively with M sulfuric acid, aqueous sodium hydrogen carbonate, and water. It was evaporated to a residue that gave spots having R_F 0.9 (unchanged material), 0.6, and 0.3 on t.l.c. (solvent: chloroform containing 3% of methanol). Column chromatography on silicic acid with chloroform as eluant gave compounds having R_F 0.6 (1.13 g) and R_F 0.3 (0.45 g).

The material having R_F 0.6 was benzyl 4-*O*-benzyl-2-[(benzyloxycarbonyl)-amino]-2-deoxy-β-D-glucopyranoside (**12**), m.p. 160–161° (from ethanol), $[\alpha]_D^{25}$ +107° (*c* 0.2, acetone).

Anal. Calc. for $C_{28}H_{31}NO_7$: N, 2.84. Found: N, 2.94.

Methylation of the 4-*O*-benzyl derivative (0.60 g) by the Kuhn procedure substituted the 3- and 6-hydroxyl groups and the NH group. The product (0.65 g) had $[\alpha]_D^{25}$ +99° (*c* 0.3, acetone); p.m.r. data: τ 6.49, 6.62 (2 OMe groups), 6.99, 7.01 (1 NMe). The product was chromatographed on a column of silicic acid with chloroform as eluant and hydrogenolyzed over palladium. 2-Deoxy-3,6-di-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**14**; 0.19 g) was obtained by crystallization from ethanol–ethyl acetate and had m.p. 184° dec., $[\alpha]_D^{25}$ +107° → +98° (*c* 0.2, equil., water).

Anal. Calc. for $C_9H_{20}ClNO_5$: N, 5.44. Found: N, 5.19.

The material having R_F 0.3 was benzyl 6-*O*-benzyl-2-[(benzyloxycarbonyl)-amino]-2-deoxy-α-D-glucopyranoside (**11**), m.p. 164–165° (from ethanol), $[\alpha]_D^{25}$ +123° (*c* 0.2, acetone).

Anal. Calc. for $C_{28}H_{31}NO_7$: C, 68.14; H, 6.33; N, 2.84. Found: C, 68.16, H, 6.38; N, 3.02.

Methylation of the 6-*O*-benzyl derivative (**11**, 0.35 g) by the Kuhn method gave the 3,4-di-*O*-methyl-, *N*-methyl derivative (0.39 g), $[\alpha]_D^{25}$ +114° (*c* 0.3, acetone) p.m.r. data: τ 6.50, 6.54 (2 OMe groups), 6.99, 7.02 (1 NMe). The product was chromatographed on a column of silicic acid with chloroform as eluant and hydrogenolyzed over palladium. Crystallization on the product from ethanol–ethyl acetate gave 2-deoxy-3,4-di-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**5**, 0.12 g) having m.p. 199° dec. Its p.m.r. spectrum was identical with one obtained from material synthesized unambiguously as follows.

Benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-β-D-glucopyranoside (**3**, 3.00 g) was dissolved in pyridine (20 ml) containing chlorotriphenylmethane (1.4

molar equiv.) and the solution was kept for 3 h at 100°. Water (2 ml) was added and the heating was continued for 30 min to decompose excess reagent. The product was partitioned between chloroform and water, and the chloroform solution was evaporated to a syrup that was fractionated on a column of silicic acid. Chloroform eluted triphenylcarbinol, and chloroform containing 2% of methanol eluted the 6-trityl ether (**6**, 3.31 g), $[\alpha]_D^{25}$ $-39°$ (*c* 0.2, acetone). A portion (0.60 g) was methylated by the Kuhn procedure and the product detritylated with 80% aqueous acetic acid for 1 h at 100°. Following chromatography on silicic acid under the above conditions benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-3,4-di-*O*-methyl-β-D-glucopyranoside (0.34 g), $[\alpha]_D^{25}$ $-24°$ (*c* 0.2, acetone), was isolated. Hydrogenolysis over palladium followed by crystallization of the product from ethanol–ethyl acetate gave 2-deoxy-3,4-di-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**5**, 0.13 g) having m.p. 199° dec., $[\alpha]_D^{25}$ $+120° \rightarrow +111°$ (*c* 0.2, equil., water).

Anal. Calc. for $C_9H_{20}ClNO_5$: N, 5.44. Found: N, 5.09.

2-Deoxy-3-O-methyl-2-methylamino-α-D-glucose hydrochloride (**8**). — Benzyl 4,6-*O*-benzylidene-2-[(benzyloxycarbonyl)amino]-2-deoxy-α-D-glucopyranoside (**10**, 1.0 g), which contained a trace of the β-anomer, was converted into its 3-*O*-methyl, *N*-methyl derivative (1.11 g), $[\alpha]_D^{25}$ $+63°$ (*c* 1.0, acetone) by the Kuhn methylation procedure; p.m.r. data: τ 6.43 (OMe), 6.97 (minor), 7.02 (NMe). The product was dissolved in 80% aqueous acetic acid (100 ml) and hydrolyzed for 1 h at 100°. Following evaporation, the resulting benzyl 2-[(benzyloxycarbonyl)methylamino]-2-deoxy-3-*O*-methyl-α,β-D-glucopyranoside was chromatographed on a column of silicic acid with 50:1 (v/v) chloroform–methanol as eluant. The product (0.76 g) was hydrogenolyzed over palladium, and crystallization from methanol–ethyl acetate provided 2-deoxy-3-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**8**, 0.29 g) having m.p. 191–192° dec., $[\alpha]_D^{25}$ $+103° \rightarrow +94°$ (*c* 0.2, equil., water).

Anal. Calc. for $C_8H_{18}ClNO_5$: N, 5.75. Found: N, 5.46.

2-Deoxy-6-O-methyl-2-methylamino-D-glucose hydrochloride (**7**). — Benzyl 2-deoxy-2-[(benzyloxycarbonyl)methylamino]-6-*O*-trityl-β-D-glucopyranoside (**6**, 2.00 g) was *O*-benzylated in 1:1 *N*,*N*-dimethylformamide–benzyl bromide (16 ml) by shaking with silver oxide (8 g) for 3 days. The solution was diluted with chloroform, filtered and evaporated to a mobile liquid containing benzyl bromide. This was then hydrolyzed for 1 h at 100° in 75% aqueous acetic acid (80 ml). Evaporation gave a residue that was fractionated on a column of silicic acid. Chloroform–hexane (1:1, v/v), followed by chloroform, eluted triphenylcarbinol. Further elution with chloroform gave benzyl 3,4-di-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-β-D-glucopyranoside (1.63 g), $[\alpha]_D^{25}$ $-29°$ (*c* 1.0, acetone). Methylation of a portion (1.02 g) by the Kuhn procedure gave the 6-methyl ether (1.10 g) p.m.r. data; τ 6.63 (OMe), 7.12, 7.48 (1 NMe). Hydrogenolysis of a portion (0.80 g) over palladium provided 2-deoxy-6-*O*-methyl-2-methylamino-α,β-D-glucose hydrochloride (**7**), which was chromatographed on a cellulose column with 7:1 acetone–water (v/v) as eluant. The purified hydrochloride (0.28 g) had $[\alpha]_D^{25}$ $+70°$ (*c* 0.2, water).

Anal. Calc. for $C_8H_{18}ClNO_5$: N, 5.75. Found: N, 6.28.

2-Deoxy-4-O-methyl-2-methylamino-α-D-glucose hydrochloride (**17**). — Benzyl 3-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-α-D-glucopyranoside (**13**, 0.76 g) was added to a solution of chlorotriphenylmethane (1.4 molar equivalents) in pyridine (4 ml), and the mixture was heated for 3 h at 100°. After decomposition of excess reagent with water (0.4 ml) the reaction product was isolated and fractionated on a column of silicic acid with chloroform as eluant. Triphenylcarbinol was eluted first, followed by the 6-trityl ether (**16**, 0.82 g), $[\alpha]^{25}$ +61° (*c* 0.4, acetone). Methylation by the Kuhn procedure gave the 4-methyl ether (0.87 g), p.m.r. data: τ 6.77 (OMe), 7.01, 7.13 (1 NMe), which was detritylated by heating for 1 h at 100° in 75% aqueous acetic acid. Column chromatography on silicic acid, with 1:1 (v/v) hexane–chloroform as eluant gave triphenylcarbinol and then benzyl 3-*O*-benzyl-2-[(benzyloxycarbonyl)methylamino]-2-deoxy-4-*O*-methyl-α-D-glucopyranoside (0.51 g). This compound was debenzylated by hydrogenolysis over palladium on charcoal, and crystallization from methanol–ethyl acetate gave 2-deoxy-4-*O*-methyl-2-methylamino-α-D-glucose hydrochloride (**17**, 0.19 g), m.p. 167–169°, $[\alpha]_D^{25}$ $+117° \rightarrow +101°$ (*c* 0.2, equil., water).

Anal. Calc. for $C_8H_{18}ClNO_5$: N, 5.75. Found: N, 5.73.

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